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Concurrent Heat Activation and Suppression of *Bacillus megaterium* Spore Germination

P. K. HOLMES, ELLA H. NAGS, AND HILLEL S. LEVINSON
Pioneering Research Division, U.S. Army Natick Laboratories, Natick, Massachusetts

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Spores of *Bacillus* spp. often require heating at sublethal temperatures in order to germinate at a maximal rate. Indeed, spore dormancy has been quantitated in terms of the requirement for heat activation, and has been evaluated in relation to sporulation conditions and to germination agent (Keynan, Murrell, and Halvorson, Nature **192**:1211, 1961; Levinson and Hyatt, J. Bacteriol. **87**:876, 1964).

Changes in the sporulation medium can change the heat-activation requirements of *B. megaterium* spores for germination on glucose and on L-alanine (Levinson and Hyatt, J. Bacteriol. **87**:876, 1964). We have now produced spores whose germination on L-alanine is suppressed by "activation" temperatures which accelerate their germination on glucose.

Spores of *B. megaterium* QM B1551 were produced on 0.5% Wilson's Liver Fraction "B," buffered at *p*H 7.0 with 0.11 M potassium phosphate (basal spores), or on this phosphate-buffered medium supplemented with 0.05 M D-glucose (glucose spores). Clean, lyophilized spores (1.0 mg per ml) were heated in distilled water suspension for 10 min at various temperatures, and were then incubated at 30 C in phosphate-buffered (0.05 M, *p*H 7.0) L-alanine (0.1 M) or D-glucose (0.01 M). The percentage of spores staining with 0.5% aqueous methylene blue was the index of germination.

After incubation for 2 hr with either L-alanine (Fig. 1) or D-glucose (Fig. 2), basal spores had germinated to an extent dependent upon the temperature of activation. Glucose spores, how-

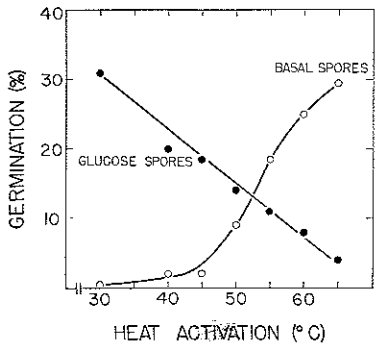

FIG. 1. *L-Alanine-induced germination of heated basal (○) and glucose-grown spores (●) of Bacillus megaterium. Heat activation for 10 min at the indicated temperatures. Germination determined after 2 hr of incubation at 30 C with 0.1 M L-alanine.*

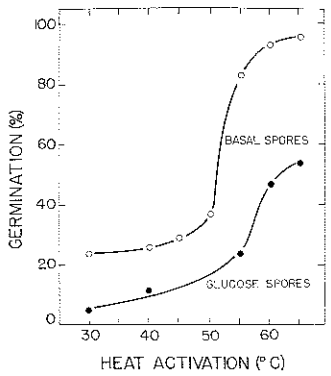

FIG. 2. *D-Glucose-induced germination of heated basal (○) and glucose-grown (●) spores of Bacillus megaterium. Heat activation for 10 min at the indicated temperatures. Germination determined after 2 hr of incubation at 30 C with 0.01 M D-glucose.*

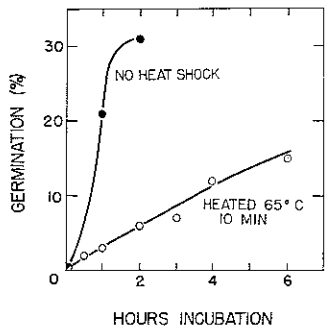

FIG. 3. *L-Alanine-induced germination of heated (○) and unheated (●) glucose-grown spores of Bacillus megaterium. After 20 hr of incubation at 30 C with 0.1 M L-alanine, both preparations showed 30% germination.*

ever, were heat-activated only for germination on glucose; their germination on L-alanine was suppressed by heat "activation."

Heating glucose spores did not decrease the total number of spores capable of germinating on L-alanine, but only lowered the rate of L-alanine-induced germination (Fig. 3) without any demonstrable lag in onset of germination. After 20 hr of incubation with L-alanine, glucose spores, whether heated or unheated, had all germinated to the same extent. Viability of glucose spores was not reduced by heating for 10 min at 65 C, as demonstrated by plate counts on nutrient agar.

The heat-induced reduction in the rate of alanine germination was not limited to glucose-grown spores. Spores grown with supplements of 0.05 M lactose or D-ribose showed similarly suppressed germination on L-alanine but not on glucose. The higher the temperature to which the carbohydrate-supplemented spores were exposed, the more slowly did they germinate on L-alanine. It has been suggested that these glucose-grown spores comprise two populations, one incapable of germination with L-alanine, and one incapable of germination with glucose. However, 100% of the glucose-grown spores germinated with a higher (100 mM) concentration of glucose. This temperature-induced dormancy, peculiar to a particular germinant, has a parallel in another system. Keynan, Issahary-Brand, and Evenchik (*In* Campbell and Halvorson [ed.], *Spores III*, American Society for Microbiology, 1965, p. 180) showed that *B. cereus* spores, heated at *p*H 1 for increasing periods of time in excess of 20 min, were increasingly dormant for germination on L-alanine plus adenosine, but were not killed. It is conceivable that a spore component, essential to L-alanine-plus-adenosine germination of *B. cereus*, was heat-sensitive at low *p*H, and that a similar component, essential to L-alanine germination of *B. megaterium*, was physiologically heat-sensitized by growing the spores with carbohydrates.